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Studies in the cytology of the Hymenomycetes, especially the Boleti

MICHAEL LEVINE

(WITH PLATES 4–8)

The discovery of sexual cell fusions in the rusts has given a great impetus to the investigation of the origin of the binucleated cells in the Basidiomycetes. It made clear that cell fusions without immediate nuclear fusions are possible and may result in an undoubted sporophyte with binucleated cells. An added importance is also given to the study of the nuclear divisions in the basidium as the stage at which chromosome reduction takes place.

Since the appearance of Brefeld's familiar observations on the origin of the carpophore of *Coprinus stercorarius*, it may be regarded as established that no specialized sex organs are necessary for the initiation of the development which leads to the formation of basidia. Brefeld (1876–77) grew the mycelium of this fungus from spores on dung decoction and described and figured two methods of carpophore formation without finding any structures visibly differentiated as sexual organs. He found that the carpophore might have its origin in a single mycelial thread or from a sclerotium, depending on the condition of the culture medium. Brefeld's results seemed for a time to settle the question as to the existence of any form of sexuality in the Basidiomycetes. The question as to the significance of the hyphal fusions in the mycelium, however, still remained open. Two types of fusions between hyphae in the Basidiomycetes have been long known,

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first the so-called clamp connections and second, the ordinary hyphal anastomoses. Such fusions are, however, well known in other groups of fungi with normal sexual reproduction and the possibility that they may have sexual significance in the Basidiomycetes seems remote.

Hoffman (1856) was the first to figure the clamp connections between hyphal cells. He clearly described the slender tube joining two adjacent cells just outside their common cross wall and gave the name "Schnallenzellen" or clamp connections to these delicate structures. Brefeld emphasized the fact that clamp connections are not permanently open, for shortly after the tube buds out, a wall appears cutting it off from the cell from which it arose. Eventually, he claims, a second wall is formed separating the clamps entirely from each of the cells which it joins. The cell which puts out the clamp connection is always the one on the apical side of the transverse wall. Harper (1902) does not agree with Brefeld on the point that the fusion tube is cut off from both cells which it joins and interprets the clamps as a means of interchange of food stuffs.

Lyman (1907) finds clamp connections arising very early. In one species, *Corticium roseo-pallens*, clamp connections are found in the germ tubes, while in *Corticium subgiganteum* no clamps at all are produced. Lyman studied artificial cultures of 75 species of Thelephoraceae, Hydnaceae, and Polyporaceae and finds accessory reproductive bodies, such as oidia in *Daedalea unicolor*, *Lenzites betulina*, *Polyporus fumosus*, *Polystictus conchifer*, *Polystictus versicolor*, and in *Corticium alutaceum;* and chlamydospores in *Lentodium squamulosum*, *Poria incrustans*, *Radulum tomentosum*, two species of *Hydnum* and one of *Phlebia*, *Corticium vagum*, *C. effuscatum*, and *Michenera artocreas*. Bulbils composed of a mass of hyphae were found in cultures of *Corticium alutaceum*. It would be extremely interesting, in view of the early origin of binucleated cells in the mycelium of many species, to know the number of nuclei in each of these types of asexual spores as an indication of whether they are to be reckoned with the gametophyte or sporophyte generation.

It has been the common view that clamp connections and hyphal anastomoses have to do with food transportation etc.

R. Hartig (1885) however believed that such cell unions resemble a sexual act and he compared these phenomena with the fusions in the Conjugatae. He traced the development of *Merulius lacrymans* from spore to spore and describes hyphal anastomoses and clamp connections as common in its mycelium and crustlike carpophore. Meyer (1896–1902) describes the formation of hyphal anastomoses in *Hypomyces rosellus*. He states that when one hyphal cell comes in contact with another a fusion may take place. The walls at the point of contact disappear and the protoplasm of the two unite. Soon afterward a new wall is formed which only incompletely separates the two cells. Meyer believes that the fusions of hyphae by anastomoses, involving the fusion of plasms not closely related, may have the same effect as cytoplasmic fusion in a normal fertilization. In view of the well established fusions found in the rusts, Voss' claim (1903) that clamp connections and hyphal anastomoses are also present in this group has considerable interest. His observations, so far however, have not been confirmed.

Ordinary pit connections for the transfer of food materials are undoubtedly present in the cross walls of the hyphae of all the higher fungi. Strasburger, 1884, maintains for *Agaricus campestris*, that the cells in the stipe show so-called protoplasmic continuity.

Miss Wakefield's (1909) observations on the conditions governing the production of the carpophore in *Schizophyllum commune* and *Stereum purpureum* are quite in harmony with the view that the origin of the carpophore in the Hymenomycetes is in no way associated with a sexual act. She finds that the production of carpophores depends upon the rate of transpiration. Thus carpophores are produced when transpiration is slow while none are formed when evaporation is too rapid.

The determination of the number of nuclei in the hyphal cells and the more exact study of their behavior has led to quite new conceptions as to the presence of sex in the Basidiomycetes. De Bary (1866) first observed the nucleus of the basidium in *Corticium amorphum*. Strasburger (1884) using alcohol and alum haematoxylin was able to demonstrate, beyond a doubt, that there are nuclei in the hyphal cells of *Psalliota campestris* and in

the basidium of *Russula rubra*, and Rosenvinge (1887) extended this conclusion to thirty-five species of Basidiomycetes. Rosen (1892) working on material from *Lepiota mucida* first observed nuclear fusions in the basidium. He concluded, however, that the secondary nucleus of the basidium resulted from the fusion of six or eight nuclei coming from the hyphae of the lamellae.

Wager (1893–'4–'9) gave us the basis for most of our present day conceptions of the nuclear phenomena in the basidium. He followed Rosen in the conception that more than two nuclei may fuse in the formation of the secondary nucleus of the basidium (see table, p. 164). He even suggests the probability of a number of nuclear fusions occurring before the primary nuclei pass into the basidium. Wager observed that in the fusion of the nuclei their chromatic reticula become intermingled and that the nucleoles finally fuse. He describes the nuclei of the basidium as having the typical structures found in higher plants, such as, chromatin, linin, differentially stained nucleoles, nuclear membranes, etc.; and was able to make out karyokinetic division figures. How the spindle is formed Wager could not make out, but believes that it comes in some fashion from an archoplasmic body. The chromosomes are six to eight in number.

Dangeard (1895) was the first to establish the fact that only two nuclei fuse to form the secondary nucleus in the basidium of *Tremella mesenterica*, *Dacryomyces deliquescens*, *Calocera viscosa*, *Craterellus sinuosus*, *Bovista plumbea*, *Nyctalis parasitica*, *Hydnum repandum*, and *Polyporus versicolor*. He was the first also to affirm the sexual nature of this nuclear fusion in the basidium as well as in the ascus, the teleutospore, and the spore of the smuts. On these facts he bases his well-known doctrine that the Ustilagineae, Uredineae, Basidiomycetes, and Ascomycetes have a sexuality which is essentially equivalent to that of the higher animals and plants. Dangeard claims that the young ascus, the young basidium, the teleutospore, and the smut spore are oögones and develop in one of the following ways. In the first case, the egg germinates by producing a promycelial outgrowth which produces sporidia endogenously as in the ascus, or exogenously as on the promycelium of the teleutospore and smut spore. In the second case the egg becomes segmented into a number of

parts, by transverse or vertical walls, as in the Protobasidiomycetes. In the last case the egg neither divides nor becomes elongated but produces sporidia on sterigmata.

In *Tremella Genistae*, *Dacryomyces chrysocomus*, *D. deliquescens*, and *Polyporus annosus*, Istvanffi (1895) claims that there is no nuclear fusion in the basidium. He further observed that there are two generations of spores formed on the basidia of *Dacryomyces chrysocomus* and *D. deliquescens*. On this point he has been confirmed by both Juel and Maire, while Dangeard holds that in these forms the secondary nucleus divides only once and only one generation of spores is formed. Istvanffi also describes the formation of two generations of spores from the basidia of *Hydnangium carneum*. He also found uninucleated oidia and chlamydospores in *Nyctalis asterophora*, *Psathyra spadiceo-grisea*, *Stropharia melasperma*, *Galera tenera*, and *Collybia tuberosa*, which would suggest the existence of a fairly long gametophytic stage, which is capable of reproducing itself asexually in these forms. In *Merulius fugax* he finds that the mycelium is not septate.

Juel (1898) points out that Brefeld's distinction between Protobasidiomycetes and Autobasidiomycetes is supported by cytological evidence since the long axis of the primary spindle is either regularly parallel or perpendicular to the long axis of the basidium in the two groups. My own observations as described below do not show any such constancy in the position of the spindle in the basidium of the Boleti. Juel finds from six to eight chromosomes, which vary in size, in the basidia of *Auricularia mesenterica*, and *Exidia truncata*.

Maire (1902), while he emphasizes the existence of an alternation of generations in the Basidiomycetes, makes no contribution to the solution of the question as to the point of origin of the sporophyte. He claims that the cells of the stipe and pileus become multinucleated (table, p. 164) by amitotic division. He found, however, that the young mycelium of *Coprinus radiatus* has uninucleated, cells and produces uninucleated oidia. He shows clearly that the nuclear fusion in the basidium is not a true fecundation and proposes to call it a "mixie," pointing out that the morphological equivalent of sexual cell fusion must come at the origin of the binucleated condition.

The basidium is a spore mother cell and not an egg and the nuclear fusion in it is associated with the phenomena of chromosome reduction. Maire describes and figures a synapsis stage in the prophases of the first division and gives good figures of centrosomes, polar asters, etc., in a number of forms. In *Scleroderma vulgare* he found a deeply staining granule on the wall of the resting nucleus, which he believes to be a centrosome. As to the number of chromosomes, Maire is plainly in error. His figures showing two chromosomes are due to poor fixations and the true chromosomes are undoubtedly shown in his protochromosomes. He first observed the appearance of cytoplasmic threads connecting the nuclei and the sterigmata at the four-nucleated stage of the basidia. He believes that the nuclei move into the sterigmata by the contraction of the threads and thus finally reach the spore. Maire observed this process of nuclear migration in several agarics, a boletus, and a puff ball.

Maire also discovered the interesting and as yet unexplained abnormalities of the basidia of *Hygrophorus conicus* and *H. ceraceus*. Their subhymenial cells and young basidia are uninucleated. The nucleus in the basidium, which is bisterigmatic, divides, forming two nuclei which migrate into the spores. Occasionally one of the nuclei in the basidium divides before entering the spore, in this case the two nuclei migrate to one spore while the undivided nucleus goes to the other spore where it divides. In *Clavaria rugosa* and *Cantharellus cinereus* he observed as many as three divisions of the secondary nucleus. In the latter species a variable number of spores are formed, from one to five, irrespective of the number of nuclei.

The observations of Wager, Juel, and Maire, as to the appearance of the spindles, astral rays, centrosomes, etc., were confirmed in the main by Ruhland (1901). He describes the nuclear phenomena in a number of Basidiomycetes (see table, p. 164). He denies that four spores are ever formed on the basidium of *Hydnangium carneum*, although the phenomena leading up to the four-nucleated stage are quite regular. He claims that either one or two spores may be formed. When two spores are formed two nuclei pass into each. In case only one spore is formed all four nuclei migrate into it.

Strands connecting nuclei and sterigmata have been observed also by Petri (1902) and Van Bambeke (1903). Petri holds that these fibers are extensions of the nuclear membranes. He counts five to six chromosomes in the first division; Van Bambeke agrees with Maire as to the number of chromosomes in the Basidiomycetes. Harper (1902) shows the presence of six to eight chromosomes on the spindle of *Hypochnus subtilis*. In this species he found all the cells of the thin filmy subhymenial layer binucleated.

Miss Nichols (1904) finds binucleated cells in the rhizomorphs of *Hypholoma perplexum*, *Crepidotus*, *Corticium lilacino-fuscum*, *Dictyophora duplicata*, *Poria*, *Pholiota praecox*, *Lepiota naucina* and *Lycoperdon pyriforme*, and holds that there is in *Hypholoma perplexum* an uninterrupted series of binucleated cells from the rhizomorphs through the stipe, pileus, trama, and subhymenium to the basidium. The germ tubes from spores of this fungus and *Coprinus ephemerus*, grown in pure culture, are commonly multinucleated; although binucleated cells were found in the mycelia of *Coprinus ephemerus* twenty-four hours old.

Fries (1911[1]) figures and describes synapsis, longitudinal splitting of the spirem, spindles with centrosomes and astral rays in the basidium of *Nidularia pisiformis*. He holds that on the primary spindle six to eight chromosomes can be seen. In the second division the chromosomes in the equatorial plate are four in number while the number seen at the poles is but two. He observed also the strands connecting the nuclei and sterigmata and describes granules which he associates with centrosomes, on the walls of the basidium at the points where the sterigmata are to appear. Fries (1911[2]) also confirms *in toto* Maire's observations on the nuclear phenomena in *Hygrophorus conicus* and holds that only one nucleus is found in the subhymenial cell and young basidium. Kniep (1911) grew the mycelium of *Armillaria mellea* from spores in a gelatin medium, and reports that its mycelial cells are uninucleated throughout. He figures basidia formed directly from this uninucleated mycelium without the development of a carpophore. Whether the bodies Kniep figures as basidia are really these organs seems doubtful.

Recent investigators have devoted much attention to the

nature and function of the cystidium, and Buller and Knoll have ascribed to them hitherto unsuspected functions. Buller (1910) as a result of elaborate studies concluded that the cystidia of the Coprini act as props and emphasizes the importance of the interlamellar spaces for the dispersal of the spores. In *Inocybe asterophora* Buller describes the cystidia as excreting a mucilaginous substance. Patouillard (1887), Massee (1887, 1894, 1904, 1906), Istvanffi (1896), Topin (1901), Maire (1910), and Demelius (1911, 1912) hold that the cystidia are organs of excretion or are in some way related to the functions of excretion. Maire (1910) holds with Topin (1901) that the function of the cystidium varies with the stage of its development. In its young stage the cystidium is a storage organ of reserve food for the hymenium. Ultimately the cystidium becomes an excretory organ. Massee (1887) holds that the cystidia of the gill-bearing fungi are the terminal cells of laticiferous vessels. Their contents escape through a nipple-like filiform attentuation at the apex of the cystidium. Miss Demelius (1911, 1912) is of the opinion that the cystidia serve to protect the fungus from the invasion of insects. She emphasizes their variability in form, as in *Collybia radicata*, which has spherical, spindle-shaped and finger-like cystidia upon which excretion products may or may not appear.

Knoll (1912) endeavors to prove that the cystidia are one-celled hydathodes comparable to the active water-excreting cells in the epidermis of the phanerogams. He maintains that trichomhydathodes, as he calls them, are also found on all parts of the carpophore as well as in the hymenium. They are definitely shaped cells having a slender foot and a much expanded middle region while the upper part forms a sort of neck. The excretion forms a spherical drop at the apex. The fluid excreted contains a jelly which remains after the water has evaporated. Besides water, Knoll believes that the cystidia excrete by-products of metabolism and so accounts for the appearance of crystals of calcium oxalate on their tops. He also holds that the hydathodes may have a protective function in the case of fungi with exposed hymenial surfaces. For Knoll, the cystidia of the Coprini are aberrant types whose true function remains obscure.

MATERIALS AND METHODS

Spores of *Pholiota praecox* and numerous Boleti were collected by placing the pilei on circular sheets of filter paper and covering the whole with a bell jar. The spore prints were kept in sterilized Petri dishes. Miss Ferguson's (1902) method of collecting spores was also used. The Petri dishes with the spore prints were kept in an ordinary refrigerator until needed. A great number of culture media were tried; among those which gave the best results were string beans, and fresh horse manure prepared in a manner similar to that described by Duggar (1901). Cherry agar was prepared by boiling 250 gms. of fresh cherries in a liter of water. The cherries on becoming soft were strained through a cheese cloth and the skins, pits, and stalks were discarded. The juice of the cherries was again boiled with 15–20 gms. of agar agar and the decoction was then filtered and sterilized in an autoclave. Malt-beef agar was made by adding 25 gms. of Loeflund's malt and 25 gms. of Liebig's beef extract to a liter of water. This was then mixed with 15–20 gms. of dissolved agar agar and boiled and neutralized with n/1000 NaOH. The solution was then filtered and sterilized.

The spores germinated best in the malt-beef medium. In the case of *Pholiota praecox* 65 per cent of the spores germinated in this medium. Cherry and malt-beef agars were used for the propagation of the mycelia of several Polypores. The cultures were kept in dark boxes at temperatures varying from 23°–30° C. The early stages of spore germination from cultures in Van Tieghem's cells were killed and fixed to slides by a modification of Harper's (1899) and Lutman's (1910) methods. The cover glass on which the culture is grown is removed from the ring and a few drops of the fixing solution are added. The cover glass with the culture is then inverted over slides covered with a film of albumen fixative and gently tapped till part of the drop falls on it. The spores in the drop become fixed to the slide by the coagulation of the albumen. The slide is then set aside till the liquid partly evaporates. The preparation is hardened by pouring graded alcohol over it, and it is then ready for staining. More fixing solution may be added to the culture drop and the process repeated till all the spores have been removed. A minimum of disturbance of the delicate germ tubes is achieved by this method.

Older mycelia of *Pholiota praecox* were obtained by sowing the spores in Petri dishes partly filled with bean, cherry, or malt agar. These were kept in the dark. After three days sufficient growth results to make the fungus visible to the naked eye. The cultures were then transferred to test tubes, from which subsequent cultures were made.

Mycelia of *Polyporus adustus*, *P. betulinus*, *P. destructor*, *P. versicolor*, *Collybia velutipes*, and *Coniophora cerebella* were obtained in pure culture from the Association Internationale des Botanistes. These were propagated by transferring small pieces (2–5 mm. sq.) of the mycelium to Petri dishes with malt-beef, cherry, and bean agars. To secure perfect penetration of the fixative in the case of mycelia, the method described by Miss Nichols (1904) was used.

Most of my material was collected in the vicinity of New York City. A number of Boleti were also sent to me from Woods Hole. In all, the carpophores of twenty-four species of Boleti and three species of Polypores were studied. Of these *Boletus granulatus*, *B. castaneus*, *B. albellus*, *B. versipellis*, *B. vermiculosus*, *B. glabellus*, *B. chrysenteron*, *B. indecisus*, and *B. pallidus* were the more favorable for cytological work on the mature carpophore. I have not succeeded in germinating the spores of any of the Boleti.

Flemming's weaker solutions gave the best results, although Merkel's, Juel's, and Bouin's gave fair fixations. Flemming's triple stain was used. Heidenhain's iron haematoxylin also gave favorable results.

To Prof. R. A. Harper, on whose advice this work was undertaken, I wish to extend many thanks for his kind suggestions and criticisms.

SPORE GERMINATION AND MYCELIA

As noted above, the question as to the origin of the binucleated cells in the higher Basidiomycetes remains still unsettled. I find that the spores of *Pholiota praecox* germinate readily and I have studied the germ tubes and young mycelium with reference to this question. Spores from spore prints, obtained in the manner described above, were sown in Van Tieghem's cells with bean, malt-beef, and dung decoctions.

Within six hours, 20 to 30 per cent of the spores will germinate, and in general, there seem to be only slight differences between cultures kept in the dark and those exposed to the light. At the end of twenty-four hours, however, 60 to 70 per cent of the spores had germinated in malt-beef decoctions while only 40 to 50 per cent had germinated in dung decoction. The spores of *Pholiota praecox* in germinating do not swell or burst but push out a dense globular bud at the apical end, opposite the point of attachment. Germinating spores, at intervals ranging from six to twelve hours, were transferred from the Van Tieghem cells to slides by the modified stippling method, described above. They were then stained with Flemming's triple stain. The globular bud that first appears from the germinating spore is very dense and at first contains no nuclei. It grows rapidly into an ordinary germ tube and a nucleus appears in it, which is soon followed by another. I have not seen nuclear division figures at this stage, but soon two, four, and more nuclei can be found in the germ tube lying near the spore. In cultures fifteen hours old (PL. 4, FIG. 1) the germ tubes have branched and a large number of nuclei are present. The main germ tube is an outgrowth of the initial globular bud which is more or less permanent, and is still visible in older cultures. The cytoplasm shows a reticulated structure with larger and more or less numerous vacuoles. The nuclei are irregularly distributed through the cytoplasm and show no definitely paired arrangement. Few if any cross walls have been formed at this stage and the hyphal cells are beyond question multinucleated. The nuclei are very small, yet each one shows a distinct nuclear membrane, and a red staining nucleole, while the chromatin is granular and stains a faint blue. In the further growth of the hyphae up to the forty-eight hour stage, new branches are formed from the bulbous initial bud near the spore, as well as from the main germ tube. Septa are still scarce in these stages; none are shown in FIG. 2, PL. 4. The diameter of the nuclei is nearly equal to that of the hypha.

The lateral branches generally show several nuclei which are similar in all respects to those in the main germ tube. The cytoplasm is denser near the apical portion of the branches and fewer vacuoles appear in this region. The cells up to the forty-eight hour stage are all multinucleated.

Malt-beef agar cultures sixty-eight hours old were imbedded in paraffin and sectioned. The cultures were made directly by sowing spores in Petri dishes or the spores were allowed to germinate from twelve to twenty-four hours in the Van Tieghem cells and were then transferred to Petri dishes. Both methods gave satisfactory results. The mycelium reaches a diameter of a centimeter on the surface of the agar within three days. Entire masses of mycelium of this size, together with the agar, were cut out and immersed in fixing solutions so as to disturb the hyphae as little as possible. The hardening with alcohols must be very carefully done. If dehydration is too rapid, the agar shrivels. Considerable difficulty is encountered in staining such sections since the agar as well as the fungus takes the stain. In using the triple stain very short exposures and dilution of the orange G are necessary. Sections of material three days old show both binucleated and uninucleated cells making up the mycelium. Long multinucleated cells are also found, which are the germ tubes of spores that germinate late. Cultures of the same age do not all of course show the same stage of development. The cytoplasm (PL. 4, FIG. 3, 4) is less dense in these hyphae and stains better. The vacuoles are large and extend across the entire width of the cells. The structure of the nuclei in the uninucleated cells appears very clearly (PL. 4, FIG. 3). The chromatin is composed of delicate strands distributed irregularly through the nuclear cavity. The nuclei of the binucleated cells as shown in FIG. 4, PL. 4, are smaller but show the same structure as those in the uninucleated cells. In cultures three days old, clamp connections and hyphal anastomoses were first noticed. Lyman (1907) reports for *Corticium roseopallens* that clamp connections may be found on germ tubes immediately after spore germination. Cultures, from spores sown in Petri dishes in malt-beef agar seven days old, make a layer of mycelium covering the entire surface of the agar. The mycelium from such cultures shows considerable numbers of multinucleated cells. Their cytoplasm is dense, the vacuoles are small and they resemble the multinucleated cells in the younger cultures described. Binucleated cells similar to those observed in cultures three days old appear more frequently and uninucleated cells are also observed occasionally. The latter are long and their cytoplasm shows large

vacuoles. Clamp connections may be seen at one or both ends of the cells and are very common. At this stage small concavo-convex bodies on both sides of the cross walls of the hyphae appear. These structures stain red with safranin and are dense, homogeneous bodies. They have been interpreted by Strasburger (1884), Harper (1902), and others as indicating the presence of protoplasmic connections between the adjacent cells. Hyphal anastomoses, such as are described by R. Hartig (1885) and Meyer (1896, 1902), are also present.

For comparison, I have also studied the mycelium of *Collybia velutipes*, which grows rapidly in malt-beef and cherry agar. Miss Cool (1912) and Biffen (1899) have both studied its mycelium. I find that the cells of the mycelium are distinctly binucleated. Narrower hyphae densely filled with cytoplasm were also found in sections of this mycelium. The terminal portion of these filaments becomes divided into uninucleated cells which give rise to cylindrical uninucleated oidia similar to those described by Biffen for the same species and Miss Nichols (1904) for *Coprinus ephemerus*. Clamp connections are regularly present and in thick sections hyphal anastomoses can be also found.

The results thus obtained show in the germ tube and early stages of mycelial growth that multinucleated cells predominate in *Pholiota praecox*, while in cultures two to three days old, multinucleated, binucleated and uninucleated cells are all present. The multinucleated cells may belong to younger hyphae as noted. In older cultures binucleated cells mainly prevail although multinucleated and uninucleated cells may be found. The origin of the binucleated condition is not clear, but it is very apparent that it appears early in the vegetative hyphae and persists throughout the subsequent development of the mycelium and the carpophore.

The cells of the mature mycelium of *Collybia velutipes* are all binucleated and here the binucleated condition may be regarded as fixed for the entire subsequent development, except as in the case of the stipe or the pileus, where the nuclei may divide, forming multinucleated cells.

Cultures of four species of Polypores obtained from the Association Internationale des Botanistes were also studied. The

species were *Polyporus adustus*, *P. betulinus*, *P. destructor*, and *P. versicolor*. The first two and the last were also studied by Miss Cool (1912). The material was propagated by transferring small pieces (2–5 mm. sq.) of the mycelia from each culture to a variety of agar media. Soft malt-beef and cherry agars proved the most favorable. Parts of such mycelia were fixed in Flemming's, Juel's, Bouin's, and Hermann's solutions but the most favorable results were obtained with Flemming's weaker solutions. Clamp connections are of course abundantly present in the cells of these mycelia. I did not specially study the development of the clamps, but it is plain that the mature clamp is not cut off from the two cells which it joins, as Brefeld (1877) held for *Coprinus stercorarius*. Only one cross wall is formed separating the clamp from the cell from which it arose. In well stained preparations hemispherical pads similar to those described above are visible on both sides of this cross wall in the clamp. This perhaps indicates only a partial closing up of the opening originally present. Similar pads may be seen also on the septa between many of the hyphal cells (PL. 4, FIG. 8). Hyphal anastomoses in these mycelia are also common. The hyphae of these Polypores (PL. 5, FIG. 5, 6) are made up of a series of regularly binucleated cells.

In the mycelium of *Polyporus versicolor* (PL. 4, FIG. 8) undoubted uninucleated cells are also present but they are not common; the mycelia in cultures of *P. destructor* and *P. betulinus* (PL. 4, FIG. 7) may show non-nucleated cells which are joined to adjacent binucleated cells by hyphal anastomoses and clamps.

I have also studied the cells of *Coniophora cerebella*, also obtained from the Association Internationale des Botanistes. They are regularly binucleated; clamps and hyphal anastomoses are also present.

It is plain that the binucleated condition is fixed in all these forms long before they proceed to the formation of a carpophore.

My mycelial cultures all showed on their surfaces the familiar appearance of droplets of water, varying in size from small globules 0.5 mm. in diameter, to large ones 10 mm. in diameter. The small drops are countless while the large ones are relatively few. Regarding Knoll's (1912) contention that many of the Basidiomycetes are provided with special hair-like organs, trichome-hyda-

thodes, for the excretion of water, I may note that my microscopic sections of mycelia in the region where the water appears showed no such specialized organs for its excretion. I found no differentiated structure that could be possibly associated with such a function and I am of the opinion that all the mycelial cells can excrete water.

CARPOPHORE AND BASIDIA OF THE BOLETI

The carpophores of the Boleti because of their soft fleshy consistency and rapid growth are more favorable for cytological study than the pilei of the Polypores. An abundance of material of the common *Boletus granulatus* collected in the fall of 1911 proved favorable for fixing and staining. A large number of other forms were also studied for comparison. Pieces of the stipe, pileus, ring,* and pores were fixed with Flemming's weaker solutions and Merkel's mixture. Flemming's triple stain was used almost exclusively although a number of preparations were stained with Heidenhain's iron haematoxylin.

Longitudinal sections of all parts of the stipe, at, above, and below the ring show it is composed of an undifferentiated mass of interwoven hyphae. The hyphal cells toward the center of an old stipe are more loosely interwoven as compared with those near the periphery and form what Ruhland has appropriately called a plectenchyma. The cells vary in diameter and present an appearance in cross section similar to that figured by Harper (1902) for *Coprinus ephemerus*, although the difference in diameter is not quite so great. The cross walls show the so-called protoplasmic connections as figured by Strasburger (1884). Clamp connections on hyphal anastomoses are entirely lacking. Many cells in the plectenchyma are binucleated; the majority, however, are multinucleated. The distribution of these cells is not regular although there is a tendency for the cells in the center of the stipe to become multinucleated early. According to Maire this multinucleated condition is the result of an amitotic division of the two original nuclei in the cell, though no one has conclusively proved

* The presence or absence of a ring is the generally accepted means of separating *Boletus luteus* and *B. granulatus*. In my material, both annulate and exannulate forms, otherwise indistinguishable, were collected at the same time and place

the presence of such divisions. The cytoplasm in the cells of the stipe contains very large vacuoles. The nuclei are comparatively (PL. 4, FIG. 9) large and have typically red-stained nucleoles and blue chromatin. In very old stipes the comet-shaped nuclei figured by Ruhland also appear. The cell walls of the cells in the ring are mucilaginous and these cells may branch. They are regularly binucleated (PL. 4, FIG. 10).

The upper surface of the semiglobular pileus of *B. granulatus* is covered by a viscid layer of slime. The flesh is thick; the pores are relatively short. Small cushion-like glandular bodies are conspicuous about their mouths.

The flesh of the pileus is made up of a meshwork of intertwining hyphae with numerous air spaces. The cortical cells of the cap are similar to those in the interior, but their walls are gelatinous. In very old material the mucilaginous layer is very thick and surrounds almost all of the cells down to the pores. The cells of the flesh are short and are almost invariably binucleated (PL. 5, FIG. 11). I have found however a few cases of cells with from three to four nuclei.

In old material of *B. granulatus* the trama, the subhymenium, and the hymenium are very distinctly differentiated. The trama is composed of bundles of long parallel hyphae. The straightest filaments are found in the center, while toward the periphery they become more interwoven. The terminal branches of the hyphae of the trama may be traced directly into the subhymenium as is well shown in *B. vermiculosus*, *B. glabellus*, and *B. pallidus*. The cell walls are thick and gelatinous, and take a blue color with the triple stain. Gelatinous material may also fill the intercellular spaces. Two nuclei are found in every cell. Frequently in the nuclei of the ring (PL. 4, FIG. 10), flesh, and trama a darkly staining granule is found on the nuclear membrane, to which the chromatin of the nuclei seems to be attached. The position of this body varies; it is sometimes found lying opposite the nucleole but frequently it is near it. This body resembles the central body described by Harper (1905), for the mycelial nuclei of the mildews. The subhymenial cells are short and are binucleated. Their cell walls do not become gelatinous, and their cross walls as also those of the trama and flesh show the characteristic hemispherical pads

described above. The basidium mother cells in *B. granulatus* are formed as in all other cases by the division of the subhymenial cells. I have not been able to find nuclear or cell division figures at this stage. The mother cell divides and the outer cell of the two may become either a cystidium or a basidium. Occasionally the last nuclear division is not followed by a cell division in the basidium mother cell of *B. granulatus*. A four-nucleated cell results (PL. 6, FIG. 45), which elongates and resembles a basidium. It is however decidedly different in appearance from the four nucleated basidium found in later stages. It is slender, more deeply seated in the hymenium and the nuclei are arranged serially. The subsequent behavior of such cells was not determined.

Paraphyses have been described as elements of the hymenium since the latter part of the 18th century. According to Léveillé (1837) the term was first used by Montagne to signify a sterile basidium, but in more recent years the function of "space maker" has been attributed to them by de Bary (1887), Brefeld (1877), Fayod (1889) and Buller (1910). It seems to me that in all cases the paraphyses are really immature basidia; as is held also by Miss Demelius, who believes that some of them, at least, are, as she describes them, "derzeit nicht fertile Basidien." In the Boleti a number of basidia in similarly advanced stages are frequently found developing in close contact with each other.

The cystidia of the Boleti appear either singly or in clusters. Such clustered cystidia are not uncommon, for Patouillard (1887), Demelius (1912), and Knoll (1912) have also found them. In *B. granulatus* such clusters are covered by a gelatinous excretion and are visible, as noted above, as minute dark specks about the mouths of the pores and over the surface of the hymenium. The individual cystidium is club-shaped; the entire cluster (PL. 7, FIG. 12) forms a cushion-shaped mass. Cystidia in all stages of development may be found in a single cluster. FIG. 12 "a," PL. 7, shows very young cystidia which are approximately the size of the mature basidia. FIG. 12 "b," 12 "c," PL. 7, represent later stages of their development. The young cystidium (PL. 5, FIG. 13) shows a dense granular cytoplasm, which is somewhat fibrillar in the upper part of the cell; vacuoles are also present. The young cystidium is regularly binucleated; the nuclei are in all essentials similar to those found in the basidium.

The subhymenial cell from which the cystidium arises is proportionately larger and may be frequently traced back into a long filament of binucleated cells in the trama (PL. 5, FIG. 15). The cell wall is slightly thickened (PL. 6, FIG. 14) and is entirely covered by the mucilaginous material referred to above. The evidence is clear that the cystidia, in *B. granulatus* at least, are glandular structures. The two nuclei are small in proportion to the volume of the cell but the nucleo-cytoplasmic equilibrium is not disturbed since the increase in the size of the cell is apparently due to an increase in the metaplasmic substances which form the source of the material that the cell excretes. The mucilaginous mass stains a faint orange with the triple stain. The outer surface of the gelatinous mass becomes dense and dark in color and ultimately forms a thin pellicle over the entire gland (PL. 7, FIG. 12). The formation of mucilage does not take place through any specialized pore as Massee (1887, 1904) holds, nor is it associated with any localized region of the cystidium as Maire (1910) and Knoll (1912) contend, but apparently takes place over the entire surface of the cystidial cell. Later the cystidium begins to disintegrate. Its cytoplasm shows large vacuoles. The nuclei also begin to show signs of disintegration. The nuclear membrane becomes faint, the nucleoles and chromatin lose their affinity for stains and soon disappear. Subsequent stages show the cell wall shriveled and the nuclei and cytoplasm entirely gone. Single flask-shaped cystidia (PL. 8, FIG. 60) are found in all the species of the Boleti I have studied except *B. granulatus*. Their nuclei and cytoplasm are essentially similar to those of *B. granulatus*. Tubular cystidia are found in *B. vermiculosus*, *B. albellus*, and *B. scaber*. They are long cylindrical binucleated cells filled with a granular cytoplasm similar to that described for *B. granulatus*.

The nuclei of the basidia of the Boleti I have studied are quite favorable for cytological study. The chromatin strands stain blue and the nucleoles a bright ruby red, with the triple stain. The nucleoles have about the same size relative to that of the whole nucleus as they have in the higher plants. The cytoplasm of the young basidium (PL. 8, FIG. 46) is finely vacuolar and stains a faint orange. With the growth of the basidium the nuclei (PL. 6, FIG. 16, 17) also increase in size. At the time of fusion their di-

ameter is several times that of the nuclei in the subhymenium. The fusion stages are abundant and easily studied. The chromatin in the nuclei before fusion loses its reticulated structure and a number of strands appear (PL. 5, FIG. 70). The nuclei in fusing become appressed upon each other and the nuclear membranes disappear in the region of contact. We have thus a two-lobed stage (PL. 6, FIG. 18) which lasts for some time, showing that the surface tension does not operate to round out instantly the united masses of the fusing pair, as might be the case if they were merely vacuoles. The nuclear membranes, however, form a continuous boundary (PL. 8, FIG. 47, 48) for the combined nuclear cavities and the constriction in the plane of fusion gradually disappears. The method of union of the chromatin masses is not easy to make out. The strands soon become mingled so as to be indistinguishable as to their origin.

I am inclined to believe, however, that the union is nothing more than the approximation of the chromatin strands bringing them into close contact with each other side by side (PL. 8, FIG. 46). At any rate the chromatin masses from the primary nuclei become so intermingled as to leave no visible evidence of their two-fold origin at this stage. The nucleoles approach, come in contact with each other, and eventually fuse (PL. 8, FIG. 49), forming a proportionately larger mass.

The basidium continues to grow and larger vacuoles appear in its cytoplasm. The secondary nucleus lies in the center of the basidium and goes through a resting period. At this stage I have also observed a dense oval body which stains red, lying in various positions in the basidia of *Boletus granulatus*, *B. albellus*, *B. versipellis*, *B. indecisus*, and *Strobilomyces strobilaceus*. In *B. albellus*, this body is sometimes found in the upper part of the basidium. Radiating from it are long strands of kinoplasm. In this species (PL. 7, FIG. 61) and in *B. granulatus* I have also found a similar structure lying between the basidium wall and the nucleus. In *B. versipellis* the body lies near the nucleus and resembles more or less the archoplasmic masses figured by Wager (1894) for *Mycena galericulata*. I have not been able to connect this body with the processes of nuclear division and I have no proof that, as Wager holds, it is the origin of the centrosomes and karyokinetic figures.

The first division usually takes place in the upper part of the basidium, although the prophase stages begin while the nucleus is still near its center. The chromatin strands combine to form a long slender thread which forms a more or less irregular coil filling the nuclear cavity. The chromatin filament may become quite thick and thus resemble a post-synaptic spirem (PL. 6, FIG. 19, 20; PL. 7, FIG. 71; PL. 8, FIG. 50). I also found a parallelism of the spirem strands which suggests splitting (PL. 6, FIG. 21). My figures of these stages show a close resemblance to those of Fries (1911[1]) for *Nidularia pisiformis*. The spirem now becomes segmented as shown in FIG. 22, PL. 6, and each segment soon becomes shorter and denser. The number of these chromatic segments apparently varies and it is very difficult to count them. During nuclear division a large vacuole appears at the base of the basidium. The nucleus moves toward the apex of the basidium and the spindle figure appears. The nuclear membrane disintegrates and the chromosomes are found lying in a faintly blue-stained fibrillar spindle whose axis is transverse to the longitudinal axis of the basidium (PL. 6, FIG. 23). The nucleole is found in the cytoplasm (PL. 8, FIG. 52, 53). In some of my preparations the nuclear membrane and nucleole seem to have disappeared before or soon after the spindle is formed, while in others both are present at the equatorial plate stage (PL. 8, FIG. 51). The direction of the long axis of the spindle in the division of the primary nucleus in the Boleti does not always conform to the generally accepted view as emphasized by Juel (1897) and others. I found that while the transverse position is common there are many cases in which the primary spindle may be very decidedly inclined, at least seventy degrees to the transverse axis of the basidium (PL. 7, FIG. 62). I have observed such cases in *Boletus granulatus*, *B. chrysenteron*, *B. badius*, *B. alutarius*, and *B. albellus*. The poles of the spindle end in small red-stained centrosomes from which long streaming rays extend to the center of the basidium (PL. 8, FIG. 51, 53). The appearance of the polar asters agrees well with that shown by Maire (1902) for *B. regius*. The cones of astral rays as seen in section resemble diminutive comet tails. The degree to which the rays are developed depends upon the position of the pole in the basidium. If the pole lies on the wall few or no rays are visible.

Compare the astral rays in FIG. 72, 73, PL. 6 and FIG. 51, 53, PL. 8, with those shown in FIG. 24, 62, PL. 7 and FIG. 58, PL. 8. Well-developed polar asters are shown in my preparations of *Boletus castaneus*, *B. glabellus*, *B. vermiculosus*, *B. versipellis*, *B. chrysenteron*, *B. punctipes*, *B. griseus*, *B. subtomentosus*, and *B. cyanescens*.

The number of chromosomes is difficult to determine but in favorable sections I have been able to count from six to eight. They lie as shown by Wager (1894), Ruhland (1901), Juel (1897), Harper (1902) and others in the center of the spindle although no characteristically dense equatorial plate is formed (PL. 6, FIG. 72, 73).

The chromosomes are drawn to the poles and at the same time the spindle elongates until its ends (PL. 6, FIG. 25; PL. 8, FIG. 54, 55) touch the basidium wall. Astral rays may be still seen radiating in all directions from the point of contact. The chromosomes become densely aggregated at the poles. I have been able, however, to see distinctly, in perfect fixations, as many as five chromosomes just before they reached the poles (PL. 6, FIG. 25a). Undoubtedly the appearance of two chromosomes in the equatorial plate and diaster stages, as reported by Maire (1902) and Van Bambeke (1903), indicates fusion of the chromosomes due to imperfect fixations.

In the reconstruction of the daughter nuclei, a nuclear membrane is formed about the chromosomes. The latter begin to stream out (PL. 6, FIG. 26) forming a reticulated structure, and small nucleoles appear. The resulting nuclei resemble in all respects the mother nuclei. In FIG. 56, PL. 8, the two daughter nuclei are shown attached to the basidium wall. Faint astral rays are still visible coming from the point of contact. The old nucleole may be seen lying in the cytoplasm. This persistence of the kinoplasmic rays is conspicuous, but they disappear before the second spindle is formed. The two daughter nuclei prepare immediately for the second division. The prophases are hard to study. I have observed in *Boletus versipellis*, that the nucleoles come to lie on the side of the nucleus toward the base of the basidium (PL. 8, FIG. 57) and pass out into the cytoplasm, after the disintegration of the nuclear membrane. The spindles show centrosomes with

long astral rays. I find that in *B. versipellis* also (PL. 8, FIG. 58) the two secondary spindles may be at an angle of about 10° to the longitudinal axis of the basidium. In these division figures, considerable variation is found in the angle formed by a transverse axis of the basidium and the long axis of the spindle.

The nuclear membranes still persist at the equatorial plate stage (PL. 8, FIG. 59). In a polar view of the equatorial plate at least four distinct chromosomes can be seen. In the diaster stage however they are often seen as one or two masses (PL. 6, FIG. 28, 29). I have been able, however, to demonstrate that at the poles also there are more than two chromosomes, as is shown in FIG. 27, PL. 6. The secondary spindles may become elongated until the chromosome masses reach the wall of the basidium as in the first division, suggesting that the kinoplasmic rays are attached to the basidium wall and are pulling the chromosomes and centers to it.

The young daughter nuclei grow rapidly in size (PL. 6, FIG. 30, 31) and remain for some time attached to the basidium wall. They then begin to move downward toward the base of the basidium and it at once becomes evident that they are connected by faintly stained strands with small granules which lie on the upper wall of the basidium at the point from which they started (PL. 5, FIG. 32). The origin of these granules cannot be easily determined. From their size, color reactions, and position in the basidium it appears that they probably may be the centrosomes, which became fixed to the wall of the basidium in the process of division. The position of the centrosomes on the upper part of the basidium wall indicates the position of the future sterigmata.

According to Petri (1902) the strands are the stretched nuclear membranes. I have been able to follow the development of these structures. In the early stages of their movement the nuclei resemble the beaked nuclei (PL. 6, FIG. 33) found in the Ascomycetes during the process of spore formation. As the main body of the nucleus progresses farther from the cell wall the fibrils become longer and thicker and resemble in all respects the strands figured by Maire (1902) and Fries (1911). I believe the fibrillar strand possibly may be analogous to astral rays; though as Petri suggests they may be due to the pulling out of the nuclear membrane.

At this stage I have also found another type of fibrils in the cytoplasm. These latter run irregularly but in the main lengthwise of the basidium. It may be that they are indications of cytoplasmic streaming.

As the sterigmata bud out (PL. 6, FIG. 34, 35, 74) the centrosomes and strands are carried upward; the centrosomes remaining at the apex of the sterigma. In mature sterigmata (PL. 7, FIG. 36, 78) the granule lies at the apex and the nucleus is still attached to it by the fibrillar strands. In this stage of development the four nuclei are found at or below the middle of the basidium (PL. 5, FIG. 37) but do not show any indication of fusing as Wager holds for *Stropharia stercoraria*. A small globular mass of cytoplasm, the spore initial, now appears at the end of each sterigma (PL. 7, FIG. 38). The growth is rapid; later stages are shown in FIG. 39, 40, PL. 7. On the upper, inner surface of the spore wall the centrosome is still visible (PL. 5, FIG. 41) and from it the fibrillar strand passes down through the sterigma to the nucleus in the basidium. It seems that the centrosome marks the apex of growth for the spore as well as for the sterigma. In the spores of *B. castaneus* (PL. 7, FIG. 75) a number of fibrils radiate downward from the centrosome through the cytoplasm but the strand which runs to the nucleus is thicker than the others.

These fibrillar strands connecting the nuclei with the sterigmata and spores are present in practically all the Boleti I have studied. In the basidia of *Merulius tremellosus* I have also observed them extending from the apex of the spores to the nuclei below in the basidium. In *Polyporus brumalis* and *P. lucidus* I could trace them only from the sterigmata to the nuclei.

Simultaneously with the development of the spores the nuclei begin to move towards the sterigmata. This migration is probably the result of the contraction of the kinoplasmic fibrils as claimed by Maire (1902). In FIG. 63, PL. 7, of *Boletus albellus* the nucleus is shown part way through the sterigma. In the same spore higher up another spherical, red staining body appears. Its lower portion is attenuated forming a long, strand-like fibril which extends in the direction of the nucleus. What the nature of this body is I have not been able to determine.

The nucleus (PL. 8, FIG. 64) divides soon after entering the spore

(PL. 5, FIG. 42). I have seen division figures in the spores of *Boletus castaneus*, *B. albellus*, *B. punctipes*, *B. cyanescens*, *B. indecisus*, *B. glabellus*, *B. granulatus*, *B. chrysenteron*, *B. spectabilis*, *B. bicolor*, *B. griseus*, *B. subtomentosus*, *B. badius*, and *Strobilomyces strobilaceus*. The equatorial plate and subsequent stages come out very clearly and well-developed polar asters are present. Long astral rays can be seen radiating from the centers and extending to the ends of the spore (PL. 7, FIG. 79). The spindles are very narrow, but show clearly in the cavity of the nucleus, the nuclear membrane being stillpresent. The chromosomes are sharply differentiated from the spindle fibers (PL. 5, FIG. 76) but are so small and so massed together that their numbers cannot be definitely counted. The position of the spindles is either transverse or parallel to the long axis of the spore. The central spindle stretches in the anaphases and the chromosomes come to lie on the walls on opposite sides of the spore (PL. 8, FIG. 43, 65). The two daughter nuclei show all the essential features of the nuclei in the basidium (PL. 8, FIG. 66). In the spore of *B. albellus*, a second division occurs. The spore (PL. 8, FIG. 67) becomes very long and the two spindles which appear are similar to those previously described. The spindles may lie parallel or at right angles to each other, the latter case is shown in FIG. 68, PL. 8, where one of a pair of spindles is represented. FIG. 67, 68, PL. 8, show clearly that the number of chromosomes is greater than two, as held by Fries (1911[1]) for the first division in the spore.

In the late anaphases, the chromosomes are found at the poles and appear to be fused into one or two masses. The nuclei are reconstructed and four small daughter nuclei result. The division here is not apparently conjugate. The spindles at least are not paired side by side, though the divisions are simultaneous. In many spores, I have found three nuclei (PL. 8, FIG. 69). Two were small, while the third was larger and had probably not yet divided. It seems probable that the condition found in these spores indicates the initial stage in germination.

The karyokinetic figures in the spores of these Boleti are very distinct and suggest that further study of young mycelia will make possible the settlement of the question as to the first appearance of conjugate division and regularly binucleated cells.

Centrosomes and astral rays are strongly and typically developed. Although it is difficult to determine the exact number of chromosomes certainly more than two are found at all stages. In the first division of the basidium where the spindles are large as many as six to eight can be counted.

Aberrant types of basidia have been found in *Boletus chrysenteron*, *B. punctipes*, and *B. griseus*. The abnormality consists in the appearance of mature sterigma-like projections while the nuclei are still in the process of division. FIG. 77, PL. 5, shows a basidium with one of its four sterigmata well developed. The division figures are perfectly normal, with the exception that they are almost perpendicular to the transverse axis of the basidium.

THEORETICAL DISCUSSION

I cannot agree with Knoll that the cystidia in the Basidiomycetes are hydathodes. The cystidia of the Boleti I have studied are evidently modified basidia whose function is in some sense glandular. The quantity of material excreted is very large and in no way resembles the mucilaginous substance found about the trama cells. Just how this substance is excreted by the cystidia is not clear but in all probability it is formed just beneath the cuticle as described by Tschirch (1889) for the gland cells in the higher plants.

As I have pointed out, the excretion products in the form of a gelatinous substance may be usually found covering the entire surface of the cell. In the case of *Boletus granulatus*, where several cystidia are found together, a cushion-like gelatinous mass is formed. These masses are the "granules" (PL. 7, FIG. 12) at the mouths of the pores. Knoll admits that a mucilaginous substance accumulates on the upper part of the cystidia of *Psathyrella gracilis*, *Galera tenera*, *G. tenuissima*, *Peniophora globulosa*, *Paneolus helvolus*, and *Coprinus lagopus* and figures the cystidia of *Collybia esculenta*, *Psathyrella consimilis*, and *Inocybe trechispora* as entirely covered with it. Knoll has done nothing to disprove the contention of Lepeschkin (1906) who showed that the discharge of water from hyphal cells in general depends only upon the condition of the plasma membrane. Biffen (1899) finds that in the case of *Collybia velutipes*, watery drops may be exuded from

any part of the carpophore, particularly the pileus, and notes three different kinds of cells covered by a watery exudation. The well-known occurrence of water drops on mycelial cultures is another fact strongly against the conception of special water-excreting organs in the fungi. As I have pointed out, cytological study of the mycelial cells shows no specially differentiated organs for the excretion of water.

The mechanical function ascribed to the cystidia by Buller for the Coprini is entirely out of the question in the Boleti. The pores need no such aid to keep them open. I have noticed in many species numbers of spores embedded in the mucilaginous covering of the cystidia. It seems that in this case the cystidia rather interfere with the dispersal of the spores than assist in it.

The question as to the method of origin of the binucleated cells and the stage at which they appear in the development of the carpophore is of interest not only with reference to its bearing on the problems of the morphology and phylogeny of the Basidiomycetes but also and even more in its bearing on the whole question of the nature of sexual reproduction. If a sporophyte with cells containing *2n* chromosomes can arise by the simple omission of a cell division in a binucleated cell and if this process can occur at various points in a mycelium either simultaneously or over quite a period of development the fact is of prime significance in the interpretation of gametic unions of the more typical sort. As noted above, the absence of differentiated sex organs at the initiation of the carpophore must be taken as an established fact, but the possibility perhaps still remains that the binucleated cells have their origin by the migration of nuclei through clamp connections or hyphal anastomoses. Meyer (1896, 1902) and R. Hartig (1885) have argued on general grounds that such cytoplasmic fusions may have some sexual significance. The observations of Lutman (1910) and Rawitscher (1912) that in the smuts the nuclei do migrate through such connecting tubes are certainly suggestive.

Voss' (1902) claim that clamp connections are present in the rusts, if confirmed, must be certainly regarded as good evidence against their function as conjugation tubes, since sexual fusions of another type are present in the rusts. Voss' observations,

however, are not generally accepted, and his figures are inconclusive. My own studies on the nature of the clamps and hyphal fusions are not conclusive, on this point. As described above, I have observed clamp connections and hyphal anastomoses in cultures three days old, but nothing that would clearly indicate nuclear migrations, though in some cases I have found empty cells adjacent to binucleated cells.

It is well established that the binucleated cells do not arise as carpophore initials. They are present long before the carpophores appear and the stimuli leading to the production of the latter seem to be vegetative and environmental.

In order to bring out clearly the stages at which binucleated sporophytic cells are found I have tabulated all the available data as to the number of nuclei in the cells of the mycelia, rhizomorphs, carpophores, etc., of the Basidiomycetes so far studied. Twenty-seven species have been described as having regularly binucleated cells in their mycelia and rhizomorphs. The data show rather clearly that the binucleated condition does not originate at any definite point but may arise anywhere in the mycelium. The germ tubes (PL. 4, FIG. 1, 2) are generally multinucleated. The young mycelium has multinucleated cells (PL. 4, FIG. 3, 4) although binucleated and uninucleated cells are also found. Miss Nichols does not believe that there are conjugate divisions in the germ tube but positive observations as to the occurrence of conjugate divisions are sadly lacking. As noted above, I have found no case of conjugate division in the material I studied.

As shown in the table, oidia and chlamydospores are reported by Istvanffi (1895), Biffen (1899), Maire (1902), and Nichols (1904), for many forms. I have studied such asexual spores on the mycelia of *Collybia velutipes*. The evidence seems to be that these spores are usually uninucleated and it would seem natural that the mycelia from which they arise should consist of uninucleated cells. Such spores are certainly not so common in the Basidiomycetes as they are in the Ascomycetes. They probably should be regarded as the asexual reproductive bodies of a gametophyte stage. As yet no binucleated spores from binucleated hyphal cells like the uredospores of the rusts have been found in the Basidiomycetes.

NUMBER OF NUCLEI IN THE CELLS OF THE BASIDIOMYCETES*

Observer	Species	Spore	Germ tube	Mycelium	Rhizomorph	Stipe	Pileus	Trama	Subhymenium	Young basidium	Carpophore
	AURICULARIACEAE										
Istvanffi	*Auricularia Auricula-Judae* L.	1 o.									
Sappin-Trouffy	" " "						2	2		2	2
Maire	" *mesenterica* Dicks	1		2						2	2
	TREMELLACEAE										
Juel	*Exidia truncata* Fr.	1								2	
Maire	*Sebacina effusa* Bref.			2						2	
Maire	" *quercina* Vuill.			2							
Maire	*Tremella Genistae* Lib.									2	
Maire	*Vuilleminia comedens* (Nees) R. Maire									2	2
	DACRYOMYCETACEAE										
Maire	*Calocera cornea*	2							2	2	2
Dangeard	" *viscosa*	1		3–4					2	2	2
Dangeard	*Dacryomyces deliquescens*	2 c. 1		2						2	
Istvanffi	" "	1 c.									
Juel	" "	1								2	
Maire	" "	2 o. 1								2	2
Perrot	*Guepinia helvelloides* Fr.									2	2
Maire	" *rufa* (Jacq.) Pat.	1								2	2
	EXOBASIDIACEAE										
Maire	*Exobasidium Andromedae* Karst.	1							2	2	
Maire	*Hypochnus Sambuci* Pers.			2					2	2	
Harper	" *subtilis*	1		2					2	2	

* A number of species were omitted where the author merely reported the number of nuclei in the basidium.

NUMBER OF NUCLEI IN THE CELLS OF THE BASIDIOMYCETES.—*Continued*

Observer	Species	Spore	Germ tube	Mycelium	Rhizomorph	Stipe	Pileus	Trama	Subhymenium	Young basidium	Carpophore
	THELEPHORACEAE										
Levine	*Coniophora cerebella*	.....	.....	2	.....	.....	.....	.....	.....	.....	.....
Nichols	*Corticium lilacino-fuscum*	.....	.....	.....	2	.....	.....	.....	.....	.....	.....
Maire	" *lacteum* Pers.	1	.....	2	.....	.....	.....	.....	2	2	.....
Maire	*Craterellus cornucopioides* L.	1	.....	.....	.....	.....	.....	.....	2	2	2
Rosenvinge	" "	2	.....	.....	.....	.....	.....	.....	2	1	.....
Dangeard	" *sinuosus*	1	.....	.....	.....	.....	.....	.....	2	2	.....
Maire	*Cyphella ciliata* Saut.	.....	.....	.....	.....	2	2	.....	2	2	.....
Maire	" *digitalis* A.-E.	.....	.....	.....	.....	2	2	.....	2	2	.....
Maire	*Dictyolus bryophilus* Pers.	1	.....	.....	.....	.....	.....	.....	.....	2	2
Maire	" *glaucus* Butsch.	1	.....	.....	.....	.....	.....	.....	.....	2	.....
Maire	*Peniophora quercina* (Pers.) Cooke	.....	.....	2	.....	.....	.....	.....	.....	2 cy.	.....
		1	.....	∞	.....	.....	.....	.....	2	2	.....
Maire	*Thelephora anthocephala* Bull.	.....	.....	.....	.....	.....	.....	.....	2	2	2
Maire	" *palmata* Scop.	.....	.....	.....	.....	.....	.....	.....	2	2	2
	CLAVARIACEAE										
Maire	*Clavaria grisea* Pers.	1	.....	.....	.....	.....	.....	.....	.....	2	2
Maire	" *rugosa* Bull.	1	.....	.....	.....	.....	.....	.....	6–∞	2	2
Rosenvinge	" *vermicularis* Scop.	2	.....	.....	.....	.....	.....	.....	.....	.....	1–4
Maire	*Sparassis crispa* Wulf.	1	.....	.....	.....	.....	.....	.....	.....	2	2
	HYDNACEAE										
Dangeard	*Hydnum repandum* L.	1	.....	.....	.....	.....	.....	.....	2	2	.....
Maire	" "	.....	.....	.....	.....	.....	.....	.....	2	2	.....
	POLYPORACEAE										
Maire	*Auriculariopsis ampla* (Lév.) R. Maire	.....	.....	.....	.....	.....	.....	.....	2	2	.....
Maire	*Boletus flavus*	2	.....	.....	.....	.....	.....	2	2	2	.....

NUMBER OF NUCLEI IN THE CELLS OF THE BASIDIOMYCETES.—*Continued*

Observer	Species	Spore	Germ tube	Mycelium	Rhizomorph	Stipe	Pileus	Trama	Subhymenium	Young basidium	Carpophore
	POLYPORACEAE										
Levine*	*Boletus glabellus*	2	.....	.....	.....	.....	.....	2	2	2 cy. 2	.....
Levine	" *granulatus*	2	.....	.....	.....	∞	2	2	2	2 cy. 2	.....
Levine	" *pallidus*	2	.....	.....	.....	.....	.....	2	2	2 cy. 2	.....
Maire	" *regius* Krombh.	2	.....	.....	.....	.....	.....	2	2	2 cy. 2	.....
Levine	" *vermiculosus*	2	.....	.....	.....	.....	.....	2	2	2 cy. 2	.....
Levine	" *versipellis*	2	.....	.....	.....	.....	.....	2	2	2 cy. 2	.....
Istvanffi	*Fistulina hepatica* Huds	1 c.	.....	.....	.....	.....	.....	.....	.....	.....	.....
Maire	" "	2 c.	.....	.....	.....	.....	2–4	2	2	2	.....
Maire	*Lenzites flaccida* Fr.	.....	.....	.....	.....	.....	2	2	2	2	.....
Istvanffi	*Merulius fugax* Fr.	.....	.....	∞	.....	.....	.....	.....	.....	.....	.....
Maire	" *lacrymans* (Wulf.) Pat.	1	.....	2	.....	.....	.....	.....	.....	2	.....
Levine	" *tremellosus*	.....	.....	.....	.....	.....	.....	.....	2	2	.....
Juel	*Muciporus corticola* (Fr.) Juel	.....	.....	.....	.....	.....	.....	.....	.....	2	2
Maire	*Polyporus acanthoides* Bull.	.....	.....	.....	.....	.....	2–∞	.....	.....	.....	.....
Levine	" *adustus*	.....	.....	2	.....	.....	.....	.....	.....	.....	.....
Istvanffi	" *annosus*	1 c. 1	.....	∞	.....	.....	.....	.....	.....	.....	.....
Levine	" *betulinus*	.....	.....	2	.....	.....	.....	.....	.....	.....	.....
Levine	" *destructor*	.....	.....	2	.....	.....	.....	.....	.....	.....	.....

* Boleti and Polypores in which I found binucleated spores and binucleated cells in the subhymenium and hymenium are *Boletus albellus, B. alutarius, B. badius, B. bicolor, B. castaneus, B. chrysenteron, B. cyanescens, B. felleus, B. griseus, B. indecisus, B. luridus, B. ornatipes, B. punctipes, B. scaber, B. spectabilis, B. subtomentosus, Strobilomyces strobilaceus, Polyporus brumalis, P. lucidus,* and *Merulius tremellosus.*

NUMBER OF NUCLEI IN THE CELLS OF THE BASIDIOMYCETES.—*Continued*

Observer	Species	Spore	Germ tube	Mycelium	Rhizomorph	Stipe	Pileus	Trama	Subhymenium	Young basidium	Carpophore
	POLYPORACEAE										
Dangeard	*Polyporus versicolor* L.	.....	.....	.....	.....	.....	.....	.....	2	2	.....
Levine	" "	.....	.....	2	.....	.....	.....	.....	.....	.....	.....
Nichols	*Poria*	.....	.....	.....	2	.....	.....	.....	.....	.....	.....
	AGARICACEAE										
Wager	*Amanita muscaria* L.	2	.....	.....	.....	.....	.....	.....	.....	2–3	.....
Maire	" *pantherina* D.C.	2	.....	.....	.....	.....	.....	.....	2	2	.....
Kniep	*Armillaria mellea*	1	.....	1	.....	.....	.....	.....	.....	1 M.B.	.....
Ruhland	" "	.....	.....	.....	.....	.....	.....	2	2	2	.....
Maire	*Camarophyllus virgineus* Wulf	.....	.....	.....	.....	.....	.....	2–∞	2	2	.....
Maire	*Cantharellus cibarius* L.	1	.....	.....	.....	.....	.....	2	2	2	.....
Maire	" *cinereus* Pers.	1	.....	.....	.....	2	2	.....	2	2	2
Perrot	" *infundibuliformis*	1	.....	.....	.....	.....	.....	.....	.....	2	.....
Maire	" *tubaeformis*	1	.....	.....	.....	.....	.....	.....	.....	2	.....
Perrot	*Clitocybe amethystina* Bolt.	1	.....	.....	.....	.....	.....	2	2	2	2–∞
Maire	" *aurantiaca* (Wulf.) Studer	2	.....	.....	.....	2	2	2	2	2	.....
Maire	*Clitopilus orcella*	1–2	.....	.....	.....	.....	2	2	2	2	.....
Istvanffi	*Collybia tuberosa* Fr.	1 o.	.....	1	.....	.....	.....	.....	.....	.....	.....
Maire	" "	.....	.....	2	.....	.....	.....	.....	.....	.....	.....
Biffen	" *velutipes*	1 o.	.....	.....	.....	.....	.....	.....	.....	.....	.....
Levine	" "	1 o.	.....	2	.....	.....	.....	.....	.....	.....	.....
Harper	*Coprinus ephemerus*	.....	.....	.....	.....	8 2	.....	2	2	2	.....
Nichols	" "	2	.....	8	.....	8	2	2	2	2	.....
Maire	" *radiatus*	1 o.	.....	1	.....	.....	.....	.....	.....	.....	2
Maire	" *tuberosus* Quél.	.....	.....	.....	2–∞	.....	.....	.....	.....	.....	.....
Nichols	*Crepidotus*	.....	.....	.....	2	.....	2	2	2	2	.....
Istvanffi	*Galera tenera* Sch.	1 c.	.....	.....	.....	.....	.....	.....	.....	.....	.....
Maire	*Gomphidius glutinosus* Sch.	1	.....	.....	.....	.....	.....	.....	2	2	.....

NUMBER OF NUCLEI IN THE CELLS OF THE BASIDIOMYCETES.—*Continued*

Observer	Species	Spore	Germ tube	Mycelium	Rhizomorph	Stipe	Pileus	Trama	Subhymenium	Young basidium	Carpophore
	AGARICACEAE										
Maire	*Hygrocybe miniata*	2						2	2	2	
Maire	*Hygrophorus agathosmus*	1						2	2	2	
Maire	" *ceraceus*	2						2–∞	1	1	
Fries	" *conicus*	2							1	1	
Maire	" "	2					2	2–4	1	1	
Maire	" *lucorum* Kalchbr.						2	2–∞	2	2	
Maire	*Hypholoma appendiculatum* Bull.	1						2	2	2	
Maire	" *fasciculare* Huds.	1						2	2	2	
Nichols	" *perplexum* Pk.	10. 2	∞		2	2	2	2	2	2	
Maire	" *sublateritium* Fr.						2	2	2	{ 4 cy. 2	
Maire	*Lactarius deliciosus* L.	2							2	{ 2 cy. 2	
Perrot	" "	1					2			2	
Maire	" *piperatus* L.	1							2	2	
Ruhland	*Lepiota cepaestipes*							2–4		2	
Ruhland	" *lilacino-granulosa* Henn								2	2	2
Rosen	" *mucida* Schr.					∞			2	6–8	
Nichols	" *naucina*			2							
Ruhland	*Marasmius scorodonius* Fr.									{ 4 cy. 2	
Maire	*Mycena galericulata*	2						2	2	2	
Wager	" "					∞			2	4	
Istvanffi	*Nyctalis asterophora* Fr.	1 ch.									
Maire	" " "	2 ch.						2	2	2	
Dangeard	" *parasitica*	2 ch.						2	2	2	
Maire	*Paxillus involutus*	2						2	2	2	
Maire	*Pholiota lucifera*	1							2	2	

NUMBER OF NUCLEI IN THE CELLS OF THE BASIDIOMYCETES.—*Continued*

Observer	Species	Spore	Germ tube	Mycelium	Rhizomorph	Stipe	Pileus	Trama	Subhymenium	Young basidium	Carpophore
	AGARICACEAE										
Levine	*Pholiota praecox*	.....	∞	1–2	.....	.....	.....	.....	.....	.....	.....
Nichols	" "	.....	.....	2	.....	.....	.....	.....	.....	.....	.....
Maire	*Pluteus cervinus*	.....	.....	.....	.....	.....	.....	∞	∞	2 cy. 2	.....
Lewis	*Psalliota bisporigera*	1	.....	.....	.....	.....	.....	.....	.....	2	.....
Maire	" *campestris* L.	4	.....	.....	.....	2–∞	.....	2–∞	2	2	.....
Rosen	" "	.....	.....	.....	.....	.....	.....	.....	.....	6–8	.....
Strasburger	" "	.....	.....	.....	.....	∞	.....	.....	.....	.....	.....
Strasburger	" *pratensis* Sch.	.....	.....	.....	.....	∞	.....	.....	.....	.....	.....
Istvanffi	*Psathyra spadiceo-grisea*	1–2 0.	.....	.....	.....	.....	.....	.....	.....	.....	.....
Maire	*Psathyrella crenata* Lasch.	.....	.....	1	.....	.....	.....	.....	2	2	.....
Maire	" *disseminata*	1	.....	.....	.....	2	2	2	2	2	.....
Perrot	*Russula integra* L.	.....	.....	.....	.....	.....	.....	2–∞	.....	2	.....
Maire	" *lepida*	1	.....	.....	.....	.....	.....	.....	2	2 cy. 2	.....
Strasburger	" *rubra*	.....	.....	.....	.....	.....	.....	.....	.....	1–8	.....
Istvanffi	*Stropharia melasperma*	.....	.....	1	.....	.....	.....	.....	.....	.....	.....
Maire	" *semiglobata*	1	.....	.....	.....	.....	∞	∞	2	2	.....
Wager	" *stercoraria*	2	.....	.....	.....	.....	.....	.....	.....	2	.....
	PHALLACEAE										
Nichols	*Dictyophora duplicata*	.....	.....	2	.....	.....	.....	.....	.....	.....	.....
Maire	*Phallus impudicus* L.	2	.....	.....	2–∞	.....	.....	.....	2	2	.....
	HYMENOGASTRACEAE										
Van Bambeke	*Hydnangium carneum* Wallr.	2–8	.....	1–2	.....	.....	.....	.....	2	2	.....
Istvanffi	" "	1	.....	.....	.....	.....	.....	.....	2	2	.....
Petri	" "	4	.....	1–2	.....	.....	.....	.....	2	2	.....
Ruhland	" "	1–6	.....	.....	.....	.....	.....	.....	2	2	.....

NUMBER OF NUCLEI IN THE CELLS OF THE BASIDIOMYCETES—*Continued*

Observer	Species	Spore	Germ tube	Mycelium	Rhizomorph	Stipe	Pileus	Trama	Subhymenium	Young basidium	Carpophore
	LYCOPERDACEAE										
Dangeard	*Bovista plumbea* Pers.	2	.....	.....	.....	.....	.....	.....	.....	2	.....
Maire	*Geaster fimbriatus* Fr.	2	.....	.....	.....	.....	.....	.....	2	2	.....
Maire	*Lycoperdon caelatum* Bull.	2	.....	.....	.....	.....	.....	2–∞	2	2	.....
Maire	" *excipuliforme* Scop.	2	.....	.....	.....	.....	.....	.....	2	2	2
Maire	" *gemmatum* Fl. Dan.	2	.....	.....	.....	.....	.....	.....	2	2	.....
Nichols	" *pyriforme*	.....	.....	2	.....	.....	.....	.....	.....	.....	.....
	NIDULARIACEAE										
Maire	*Cyathus hirsutus*	.....	.....	.....	.....	.....	.....	.....	2	2	2
Maire	*Nidularia globosa* Ehr.	2	.....	.....	.....	.....	.....	.....	.....	2	2
Fries	" *pisiformis* Tub.	2	.....	.....	.....	.....	.....	.....	.....	2	2
	SCLERODERMATACEAE										
Maire	*Scleroderma vulgare*	2	.....	.....	.....	.....	.....	.....	2	2	.....

o. = oidia
c. = conidia
ch. = chlamydospores
cy. = cystidia
M.B. = mycelial basidia
∞ = multinucleated

The question as to the possible morphological equivalence of the carpophore of the Basidiomycetes and the ascocarp of the Ascomycetes is a difficult one. The development of the ascocarp is in many cases at least initiated by functional or possibly non-functional sex organs. It is possible that in the apogamous Ascomycetes which have been reported as lacking an ascogone, the ascocarp may arise in the same fashion as does the carpophore in the Basidiomycetes. It is to be noted however that binucleated mycelia are not so far known to occur in these apogamous Ascomycetes.

I am of the opinion that in the Boleti at least none of the cells of the hymenium may be properly regarded as paraphyses. They are all binucleated when young and are all potentially either basidia or cystidia. That certain of these cells serve merely as "space makers" is an interpretation which appears to me to be without much proof. Certainly there are no paraphyses here which can be compared to those in the Ascomycetes. In the latter the paraphyses are typically gametophytic while in the Basidiomycetes all the elements of the hymenium are just as certainly sporophytic.

The constancy in the occurrence of a nuclear fusion followed by a double nuclear division in the basidium is now generally conceded. Uninucleated basidia such as those of *Hygrophorus conicus* reported by Maire (1902) and Fries (1911[2]) are apparently rare exceptions. The young basidia of twenty-four species of Boleti and three species of Polypores which I have examined are all binucleated. The two nuclei fuse in all cases and the resulting nucleus divides twice, forming four nuclei. These four nuclei migrate to the spores and there divide again.

It is an obvious conclusion that these two nuclear divisions in the basidium involve the reduction of the chromosome number but the small size of the nuclei makes it impossible to reach new or independent conclusions as to the nature of the reduction process. Fries (1911[1]) and Kniep (1911) hold that the first and second divisions in the basidium are respectively heterotypic and homoeotypic. But it cannot be said that their figures give any very positive evidence on the large questions here involved. My preparations show clearly (PL. 6, FIG. 27) that the number of chromo-

somes in the second division is certainly greater than two. The number which can be distinguished is variable but more than two are always found. This is true even in the case of the very small karyokinetic figures of the second division in the spore as shown in FIG. 68, PL. 8. That we have a reduction division in the basidium comparable to that found in the spore mother cells of the higher plants is hardly to be questioned on general grounds; but the diminutive size of the chromosomes makes the study of the process extremely difficult. It is certainly clear, however, that Maire's (1902) and Van Bambeke's (1903) two chromosomes in both divisions, and Fries' (1911) two chromosomes in the second division and in the division in the spores are the results of poor fixation causing the fusion of the chromosomes.

My observations on the Boleti confirm the views of Wager (1903, 1904), Juel (1897), and Harper (1902), and it cannot be doubted that Maire's protochromosomes are the true chromosomes.

Maire (1902) and more recently Fries (1911) have given extremely interesting data as to the migration of the four nuclei from the basidium into the spore. The nuclei according to their observations are drawn into the sterigmata by fibrillar cytoplasmic strands which extend from the sterigmata to the nuclei. My preparations show clearly also the kinoplasmic strands extending from the nuclei to the points of origin of the sterigmata (PL. 7, FIG. 36; PL. 5, FIG. 37). Petri held that these strands originate by stretching of the nuclear membrane. The daughter nuclei in the telophases become attached to the basidium and when they move downward in their well-known migration, the nuclear membrane is drawn out into a long fibrillar strand. The strand may be compared to a much attenuated beak on the nucleus. Another interpretation of these strands is possible. As noted in the telophases of the second division, the daughter nuclei come to lie on the wall of the basidium and apparently their positions mark the point of origin for the future sterigmata. When the nuclei migrate downward in the basidium, the centrosomes are left behind on the basidium wall. The sterigma, budding out at just this point, carries the centrosomes upward (PL. 5, FIG. 37), at its apex. The strands connecting the centrosomes and nuclei are thus carried

upward with the growth of the sterigma. This growth of the sterigma naturally involves flowage of material into it and that this movement should express itself in the formation of fibrillar strands between the nucleus and the centrosome at the apex of the sterigma is certainly to be expected and indicates the relation of the nucleus to the metabolic activities of the basidium. The strands on this view would be comparable, in general, to the kinoplasmic astral rays.

My preparations show further that when the spore buds out on the apex of the sterigma the centrosome is carried up at the apex of the growing spore. The fibrillar strands are extended also into the spore and appear running through its long axis and maintaining a continuous connection with the nuclei which now lie at the middle or towards the base of the basidium (PL. 7, FIG. 40, 75; PL. 5, FIG. 41).

It appears to be generally conceded that these strands are associated in some way with the passage of the nuclei into the spore. My observations in the Boleti certainly give good ground for the contention that they at least direct the course of the nuclei into the spores. The fact that, as I find, these strands extend through the sterigma and into and through the spore body, suggests also that they may be of significance for the apparently difficult process of getting the nucleus through the narrow neck of the sterigma.

I find nothing in the Boleti to favor Fries' (1911[1]) claim that the migrating nuclei undergo a characteristic transformation in passing into the spore. It is, perhaps, not entirely proven that these fibrillar strands are actively contractile kinoplasmic elements which pull the nuclei into the spores but the appearances in the Boleti certainly suggest such a conclusion. Such a conception is, of course, not inconsistent with the view that such strands may also constitute a system of lines of flowage of material into the young spore.

SUMMARY

1. Spores of *Pholiota praecox* germinated in malt-beef extract at room temperature produce multinucleated germ tubes. The mycelia in cultures forty-eight hours old are still composed of long multinucleated cells. In cultures three days old both uninucleated and binucleated cells are found.

2. The mycelia of *Collybia velutipes*, *Polyporus adustus*, *P. betulinus*, *P. destructor*, *P. versicolor*, and *Coniophora cerebella* propagated from old cultures are made up of long series of binucleated cells. Clamp connections, hyphal anastomoses, and the so-called protoplasmic connections are numerous in all the mycelia.

3. The cells of the mature stipe of *Boletus granulatus* are all multinucleated, while the cells of the ring are binucleated. The cells of the flesh and trama of *B. granulatus* are binucleated. The cells of the subhymenium are binucleated in all the species of Boletus studied.

4. The cystidia of the Boleti occur either singly or in small clusters forming gelatinous granules. In *B. granulatus* these cushion-shaped "granules" are abundant at the mouths of the pores and scattered over the hymenium. The individual cystidium is binucleated. It is club-shaped and is deeply seated in the hymenium. The cystidia of the Boleti appear to be in some sense glandular in their functions.

5. The nuclear phenomena in the basidium are typical in all the species of Boletus examined. Fusion of the two primary nuclei of the basidium was observed in *Boletus granulatus*, *B. versipellis*, *B. glabellus*, *B. vermiculosus*, *B. castaneus*, *B. albellus*, and *B. chrysenteron*.

6. The long axes of the spindles in both divisions are commonly transverse to the long axis of the basidium. Variations, however, appear in which the spindles are almost perpendicular to the transverse axis of the basidium. Centrosomes and well-developed astral rays are regularly present.

7. The chromosome number in the first division is from six to eight in *Boletus granulatus*, *B. castaneus*, *B. albellus*, *B. vermiculosus*, *B. versipellis*, and *B. chrysenteron*. In the second division the exact number cannot be determined. It is, however, always more than two.

8. At the end of the second division the centrosomes become attached to the walls of the basidium and the four daughter nuclei are reconstructed in close connection with them. As the nuclei move downward in the basidium they maintain their connection with the centrosomes by means of fibrillar strands which are, perhaps, analogous to astral rays. The fibrillar strands apparently pull the nuclei into the spores.

9. The centrosomes mark the points of origin for the sterigmata. They are carried up with the growth of the sterigmata and into the spores. They also apparently determine the apex of growth of the spores.

10. The spores in all the forms studied are uninucleated at first. The primary spore nucleus divides at once. The karyokinetic figures are small but very sharply differentiated with well-developed centrosomes and polar asters. In the spores of *B. albellus* a second division occurs.

11. In *Boletus chrysenteron*, *B. punctipes*, and *B. griseus*, basidia with mature sterigmata are found before the completion of the second division. Normal basidia are also present.

12. An alternation of generations comparable to that in the Uredineae is also present in the Basidiomycetes. The sporophyte begins at some indefinite point in the mycelium and extends through the development of the carpophore.

COLUMBIA UNIVERSITY, NEW YORK.

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All the figures were drawn with the aid of a camera lucida and with the Leitz 1/16 objective. Figs. 11, 14, 16–45 were made with ocular 4; distance from camera mirror to drawing board 260 mm. Figs. 1, 3, 4, 5, 6, 9, 10, 46–79 were made with ocular 4; distance from camera mirror to drawing board 170 mm. Figs. 13, 15 were made with ocular 4; distance from camera mirror to drawing board 90 mm. Fig. 7 was made with ocular 3; distance from camera mirror to drawing board 90 mm. Figs. 2, 8, 12 were made with ocular 1; distance from camera to drawing board 90 mm.

Explanation of plates 4–8

PLATE 4

Pholiota praecox.

FIG. 1. Germination tube at the end of fifteen and a half hours.

FIG. 2. Same as Fig. 1 at end of forty-eight hours.

FIG. 3. Uninucleated hyphal cells in mycelium three days old.

FIG. 4. Binucleated hyphal cells in mycelium three days old.

Polyporus betulinus.

FIG. 7. Binucleated hyphal cells from old mycelium showing clamp connections and anastomoses.

Polyporus versicolor.

FIG. 8. Binucleated hyphal cells from old mycelium showing clamp connections, anastomoses, hemispherical discs.

Boletus granulatus.

FIG. 9. Cells from the stipe.

FIG. 10. Cells from the ring.

PLATE 5

Polyporus destructor.

FIG. 5. Binucleated cells from old mycelium.

FIG. 6. Two adjacent binucleated cells with clamp connections.

Boletus granulatus.

FIG. 11. Binucleated cells from the flesh of the pileus.

FIG. 13. Young binucleated cystidium.

FIG. 15. Mature and very young cystidium showing connection with hyphal cells in the trama. Line over mature cystidium indicates surface of gelatinous excretion.

FIG. 32. Cross section of basidium showing four daughter nuclei telophase stage.

FIG. 37. Four nuclei a little below the middle of the basidium attached to the tips of the sterigmata by fibrillar strands.

FIG. 41. Basidium showing the nuclei in the sterigmata attached to the tips of the spores by fibrillar strands.

FIG. 42. Nuclear division in the spore in equatorial plate stage.

FIG. 44. Same as FIG. 42, diaster stage.

Boletus castaneus.

FIG. 70. Primary nuclei in basidium.

FIG. 76. Nuclear division in spore showing spindles with centrosomes and astral rays, equatorial plate stage.

Boletus chrysenteron.

FIG. 77. An abnormal basidium showing one of the sterigmata fully formed while the two daughter nuclei are still dividing.

PLATE 6

Boletus granulatus.

FIG. 14. Mature and young cystidia.

FIG. 16, 17. Young basidia with primary nuclei preparing to fuse.

FIG. 18. Basidium showing nuclear fusion.

FIG. 19, 20. Basidium with nucleus in spirem stage.

FIG. 21. Longitudinally split spirem.

FIG. 22. Segmented spirem.

FIG. 23. First nuclear division showing eight chromosomes, metaphase.

FIG. 25. The chromosomes moving toward the poles, anaphase.

FIG. 25*a*. The chromosomes at the poles before fusing, diaster.

FIG. 26. Cross section of basidium showing two young daughter nuclei, telophase.

FIG. 27. Diaster, second division, a number of nuclei at each pole.

FIG. 28, 29. Cross section of basidium, same as FIG. 27; chromosomes fused into two masses.

FIG. 30, 31. Early telophase of the four daughter nuclei.

FIG. 33. Four nuclei beginning to move toward base of basidium, nucleus to right is attached to centrosome on basidium wall by kinoplasmic fibers.

FIG. 34, 35. Early stages in the development of the sterigmata, fibrillar strands connect the nuclei and the tips of the sterigmata.

FIG. 45. Young four-nucleated hymenial cell in which nuclear division was not followed by cell division.

Boletus castaneus.

FIG. 72. Cross section of basidium, first division, the chromosomes massed together.

FIG. 73. Same as FIG. 72, six to eight chromosomes.

FIG. 74. Nuclei moving to base of basidium.

PLATE 7

Boletus granulatus.

FIG. 12. A cluster of cystidia forming a gelatinous granule, from the mouth of a pore.

FIG. 24. First division in the basidium, anaphase, showing a number of chromosomes, the poles lying on the basidium wall.

FIG. 36. Basidium with four nuclei attached to young sterigmata.

FIG. 38. Basidium with very young spore buds.

FIG. 39. A slightly older stage; fibrillar strands distinctly visible running through the spore body connecting centrosomes and nuclei.

FIG. 40. An older stage; fibrillar strands; nuclei moving toward the spores.

Boletus albellus.

FIG. 61. Resting nucleus with a conspicuous red-staining body lying between it and the basidium wall.

FIG. 62. First division, anaphase, with well-marked centrosomes and astral rays.

FIG. 63. Nucleus in a sterigma.

Boletus castaneus.

FIG. 71. A spirem stage.

FIG. 75. Basidium with the nuclei in the sterigmata, attached by fibers to the centrosomes at the apices of the spore.

Boletus chrysenteron.

FIG. 78. Nuclei attached to the tips of sterigmata by kinoplasmic fibers.

FIG. 79. Nuclear division in the spore; spindles with centrosomes and astral rays. Chromosomes in equatorial plate stage.

PLATE 8

Boletus granulatus.

FIG. 43. Nuclear division in spores, anaphase.

Boletus versipellis.

FIG. 46. Basidia with primary and secondary nuclei.

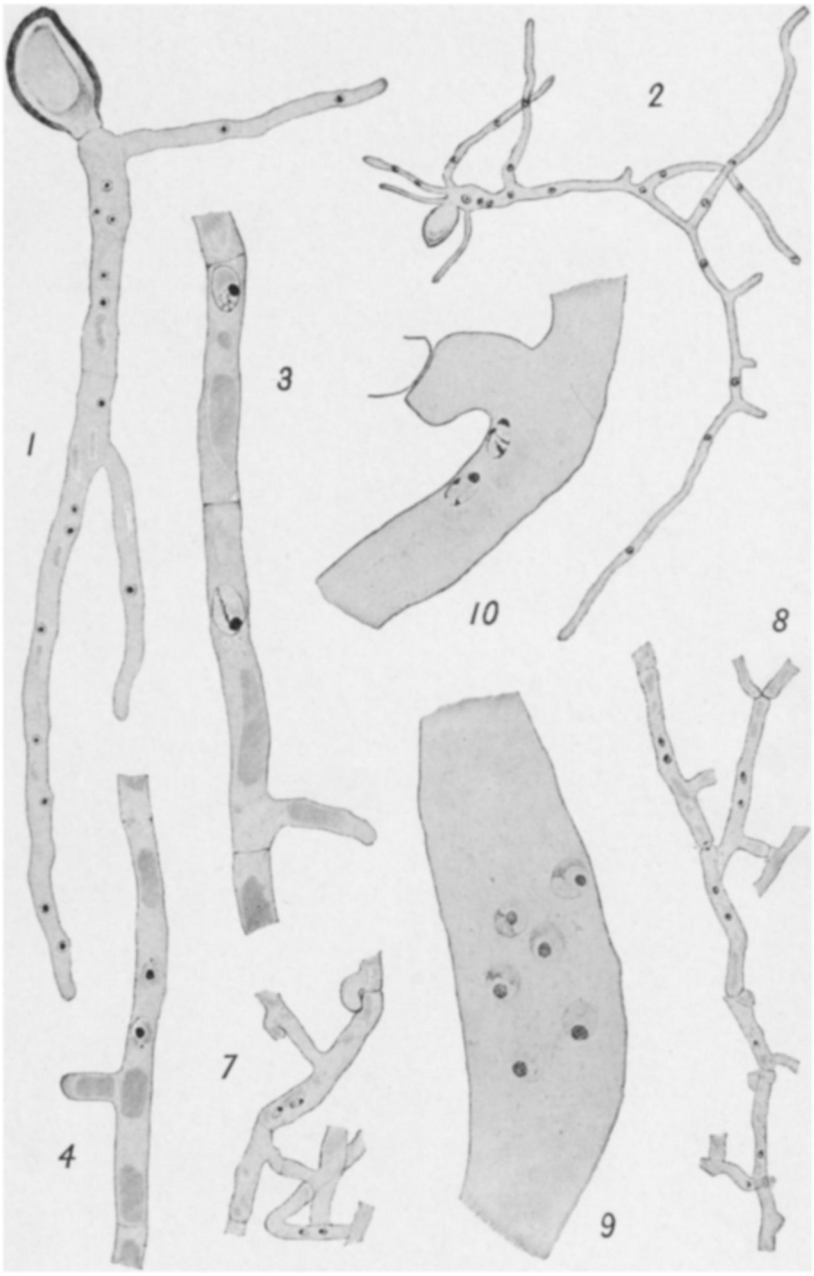
FIG. 47, 48, 49. Nuclear fusions in the basidium.

FIG. 50. Spirem stage.

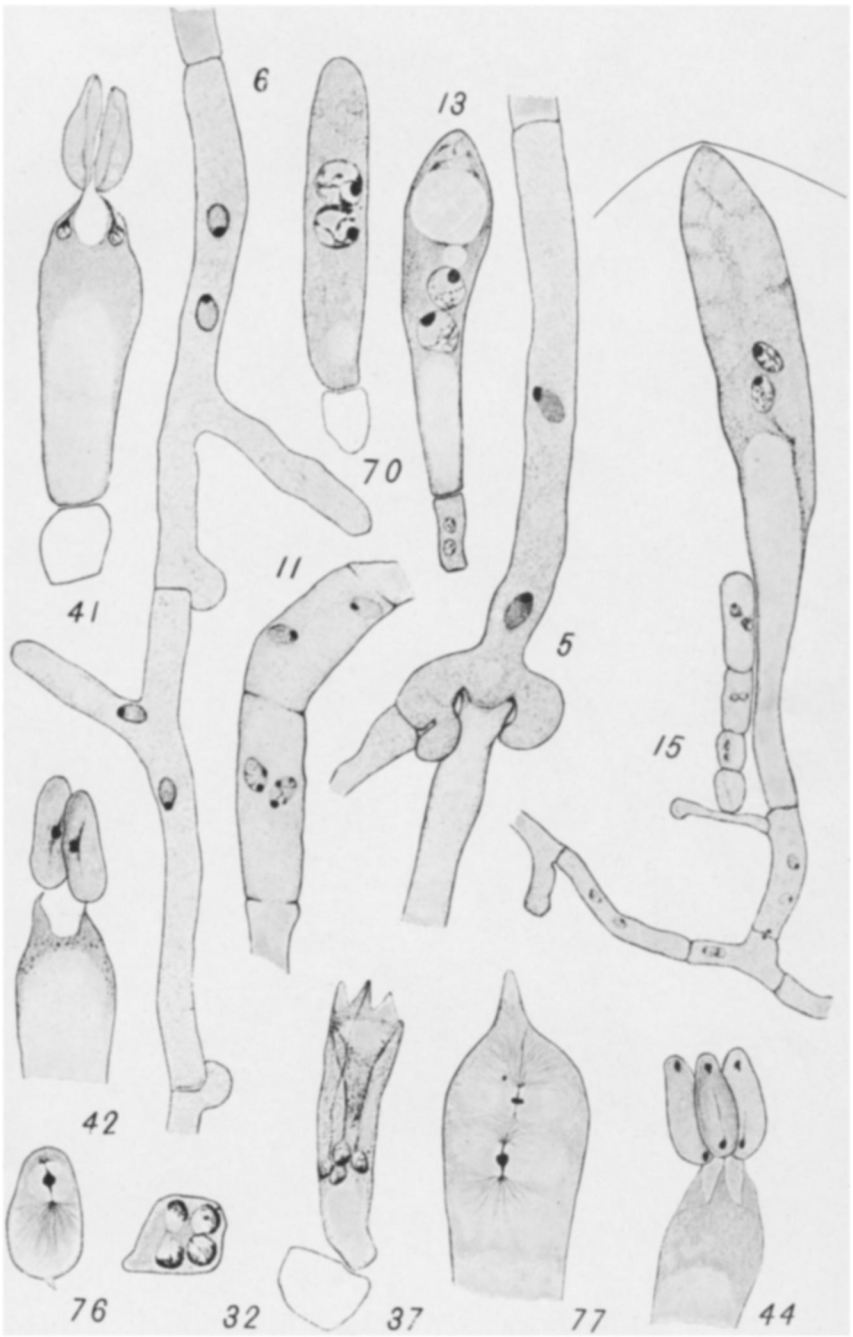
FIG. 51, 52, 53. First division in the basidium, varying appearance of the equatorial plate stage.

FIG. 54, 55. Diaster, stages of the first division.

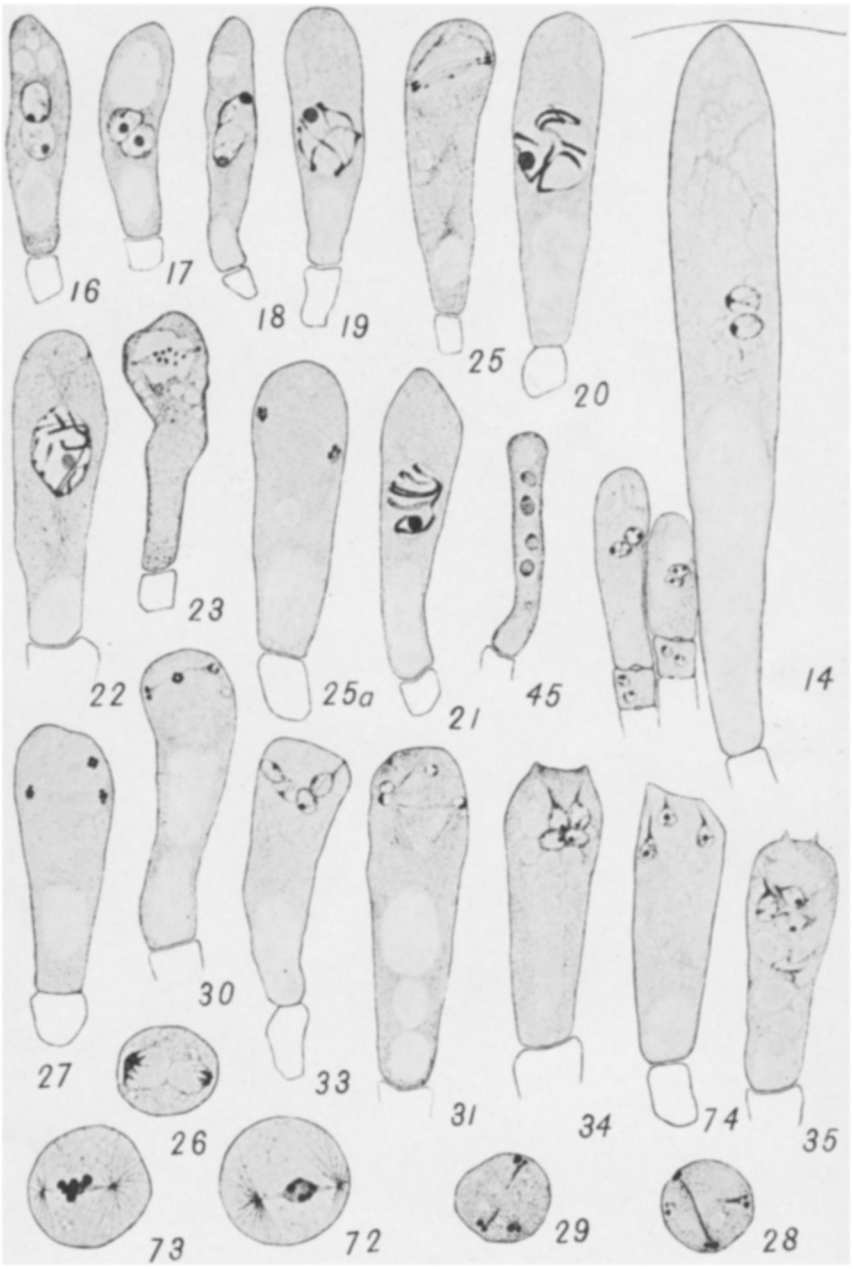
FIG. 56. Two daughter nuclei attached to wall of basidium, astral rays still present, late telophase.



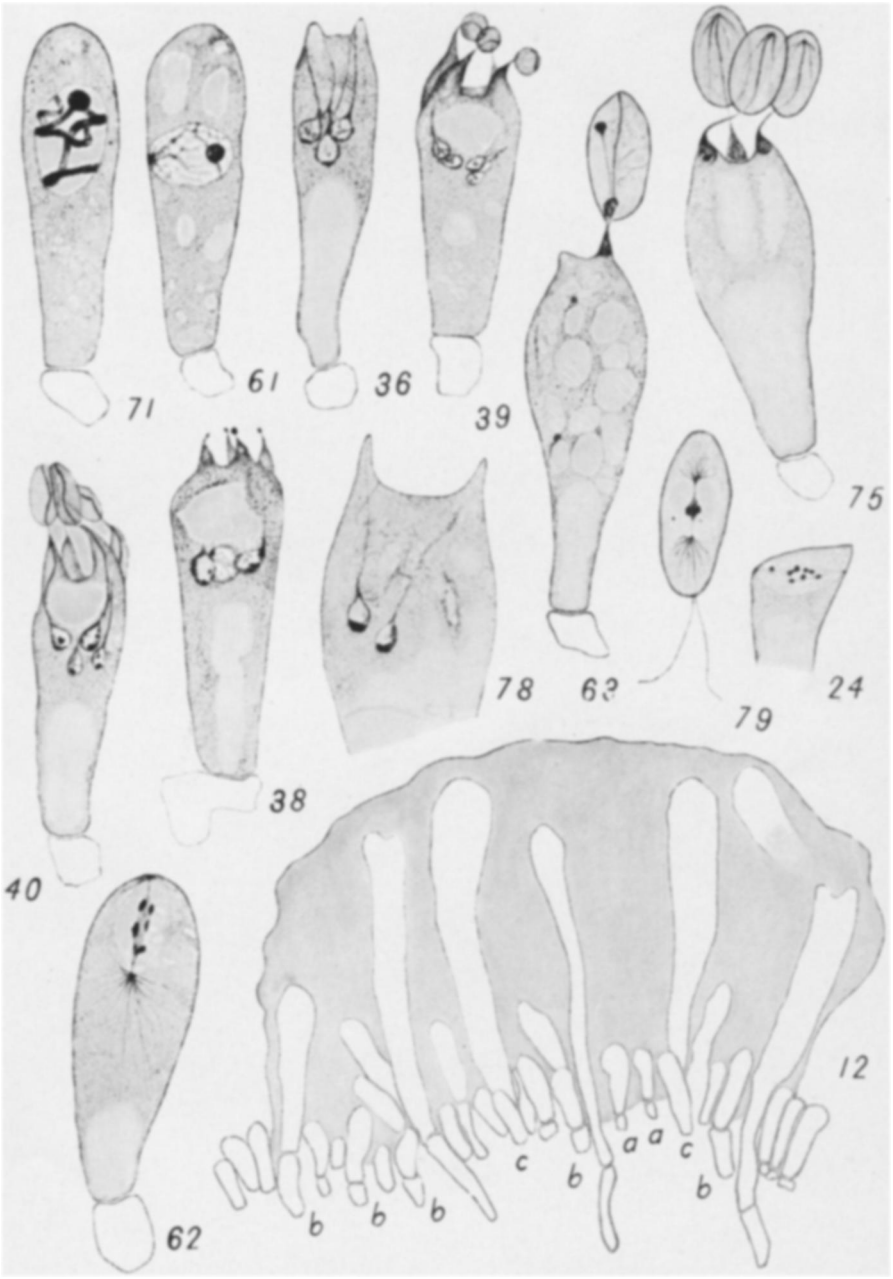
LEVINE: CYTOLOGY OF HYMENOMYCETES



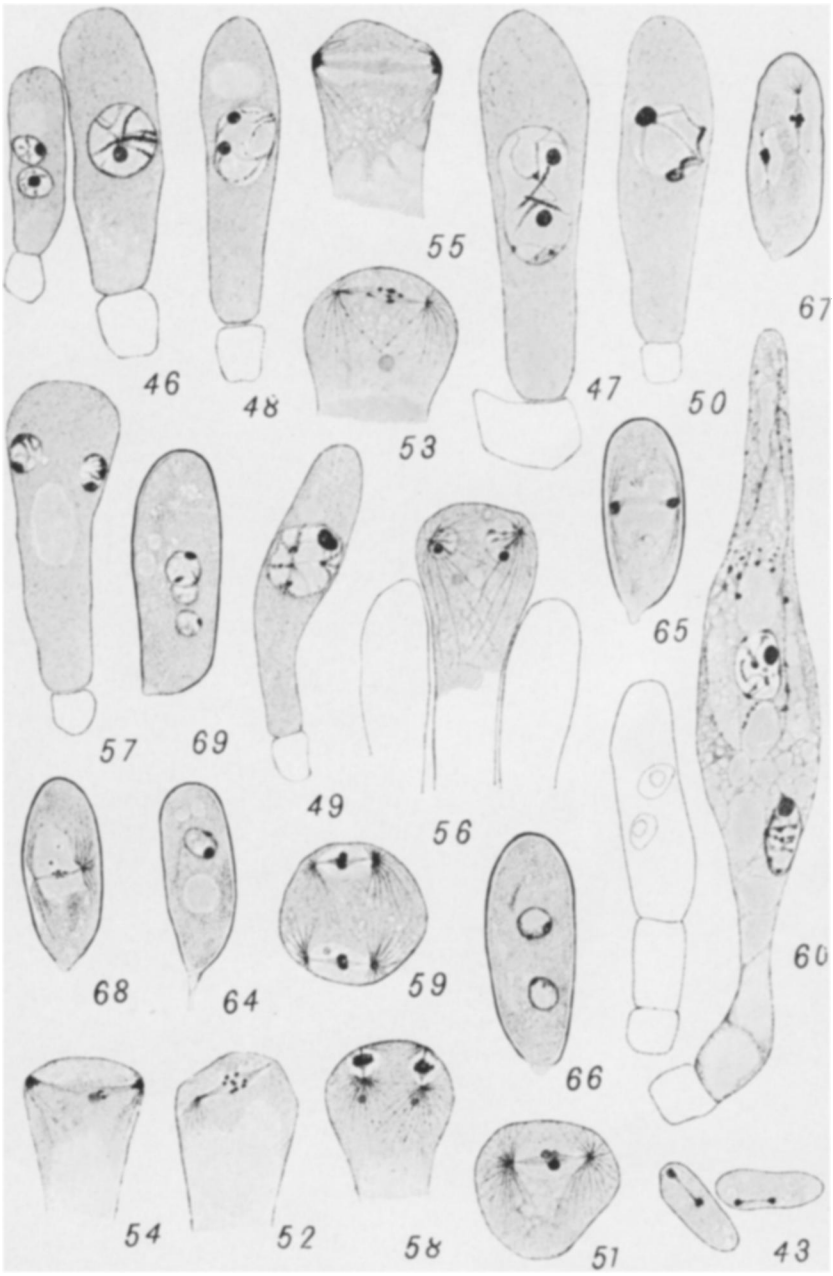
LEVINE: CYTOLOGY OF HYMENOMYCETES



LEVINE: CYTOLOGY OF HYMENOMYCETES



LEVINE: CYTOLOGY OF HYMENOMYCETES



LEVINE: CYTOLOGY OF HYMENOMYCETES

FIG. 57. A prophase stage of the second division.

FIG. 58, 59. Second division in the basidium, spindle with centrosomes and astral rays, chromosomes in equatorial plate stage.

FIG. 60. Cystidium with two sharply differentiated nuclei, to the left is a basidium.

Boletus albellus.

FIG. 64. Spore with single nucleus.

FIG. 65. First division in spore, diaster stage.

FIG. 66. Binucleated spore.

FIG. 67. Second division, spindles with centrosomes and astral rays, chromosomes in equatorial plate stage.

FIG. 68. Similar stage, only one spindle is figured; several chromosomes are shown.

FIG. 69. A spore with three nuclei.